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Impairment of Shuttlebox Avoidance Learning following Repeated Alcohol Withdrawal Episodes in Rats[1]

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BOND, N. W. *Impairment of shuttlebox avoidance learning following repeated alcohol withdrawal episodes in rats.* PHARMAC. BIOCHEM. BEHAV. 11(5) 589–591, 1979.—Rats were reduced to 70% of their free-feeding body-weights and then randomly assigned to one of three groups. An Alcohol Withdrawal group was placed on a liquid diet consisting of alcohol plus Sustagen for four 5-day periods interspersed with three 2-day periods of a liquid diet containing sucrose plus Sustagen. A Continuous Alcohol group received the alcohol diet for 20 days, followed by six days on the sucrose diet. A Sucrose Control group received the sucrose diet only and was pair-fed the amounts consumed by the Alcohol Withdrawal group. Thirty days after the diets were discontinued the rats were given 60 trials on a shuttlebox avoidance task. The results showed that the Alcohol Withdrawal group was impaired in shuttlebox avoidance when compared to the Continuous Alcohol group and Sucrose Control group.

Rats Alcohol withdrawal Liquid diets Shuttlebox Learning ability

THERE is a voluminous literature demonstrating that alcoholics display deficits in certain cognitive functions compared to nonalcoholics [7]. Further, a number of studies have established that the duration of their drinking history is related to the degree of impairment [3]. Originally, most investigators attributed these deficits to the malnutrition which frequently accompanies chronic alcoholism [11]. However, there is now evidence in animals that alcohol per se or its metabolites may also impair learning ability in the absence of malnutrition [2, 4, 6, 12, 13].

Given that alcohol is directly implicated in causing cognitive dysfunctions and that the degree of impairment is correlated with the duration of drinking history, a number of authors have sought to determine if there exists a relationship between the course of alcoholism and intellectual deterioration [1, 3, 7, 9]. For example, Ludwig and Cain [9] sought to determine whether the observed deficits are due to chronic alcohol toxicity, or alternatively to the consequences of repeated or severe alcohol withdrawal episodes. They found a slight but significant correlation between the severity and frequency of prior alcohol withdrawal experiences and cognitive dysfunction. However, since they excluded subjects who showed overt evidence of brain damage, a frequent accompaniment of alcoholism [7], their estimate is probably conservative.

A separation of the effects of chronic alcohol toxicity from the effects of repeated alcohol withdrawal reactions is extremely difficult in man. A methodology is required that allows us to distinguish the effects of the withdrawal reaction from the effects of the alcohol consumption needed to produce the withdrawal reaction. Freund [5] has provided this basic methodology by demonstrating that repeated weekly withdrawal from alcohol in mice resulted in an impairment of learning ability in a shuttle box avoidance task, after five weeks of alcohol consumption followed by 10 days of laboratory chow consumption without alcohol. Continuous alcohol consumption for the same period did not impair learning ability. However, Freund [5] did not include daily alcohol consumption or the body weights of the various groups in his report. As such, it is possible that the impairment observed in the repeated withdrawal group was due to either increased alcohol intake on its part or to malnutrition, since one correlate of alcohol withdrawal is weight loss [10]. Therefore, the present study was designed to investigate the role played by repeated withdrawals from alcohol on shuttlebox avoidance learning in rats. A Sucrose Control group pair-fed with the Alcohol Withdrawal group was employed to control for the effects of malnutrition and the parameters of the experiment were chosen so as to produce similar daily alcohol intakes in the Alcohol Withdrawal and Continuous Alcohol groups.

METHOD

Animals

Thirty-one experimentally naive male Wistar rats were employed. They were 100 days old and weighed 216–368 g. Throughout the experiment the rats were maintained in a temperature controlled room on a 12 hr light-dark cycle and were housed individually in wire cages measuring 15×24×20 cm.

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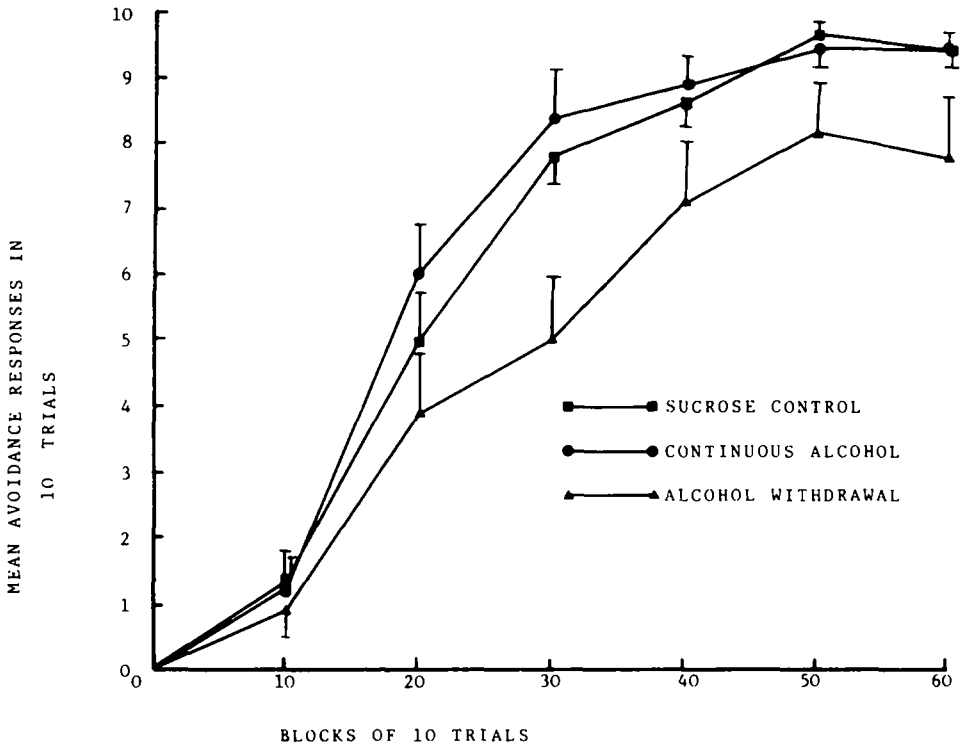

Fig. 1 Mean avoidance responses in 10-trial blocks for each group. Standard errors are indicated by the vertical lines.

Apparatus and Procedure

The rats were divided into three groups matched on body-weight. They were reduced to 70% of their pre-experimental weights and then placed on liquid diets [5]. These diets were similar to those used by Walker and Freund [12] except that Sustagen was substituted for Metrecal. That is, the Alcohol diet contained 35% ethanol-derived calories, and the Sucrose diet was identical except for the isocaloric substitution of sucrose for ethanol. Fresh diet was mixed daily.

The Alcohol Withdrawal group received the liquid diet containing ethanol and Sustagen for the first five days. They were then switched to the Sucrose diet for the next two days. This cycle was repeated until the Alcohol Withdrawal group had received the Alcohol diet for four 5-day periods interspersed with three 2-day periods on the Sucrose diet [5,10]. When on the Sucrose diet the Alcohol Withdrawal group were fed the amount they had drunk on the last day of the preceding Alcohol diet cycle.

The Continuous Alcohol group received the liquid diet containing ethanol and Sustagen for 20 days, i.e. the same number of days as the Alcohol Withdrawal group, and was then switched to the Sucrose diet for six days receiving the amount they had consumed on the last day on the Alcohol diet.

The Sucrose Control group received the liquid diet containing sucrose and Sustagen for 26 days and were pair-fed the amount drunk by the Alcohol Withdrawal group on the previous day.

Following the liquid diet phase of the experiment, all animals were maintained on ad lib dry food and water for 30 days to allow sufficient time for alcohol and its metabolites to be eliminated by the experimental animals. They were then tested in the shuttlebox avoidance apparatus.

The shuttlebox consisted of two clear perspex compartments (13 cm high × 30 cm long × 10 cm wide) separated by a black guillotine door. It was fully automated and response latencies were recorded by means of automatic printout timers. All equipment was situated in a darkened, temperature-controlled, sound-attenuated cubicle. Animals were allowed five minutes to explore the shuttlebox with the guillotine door raised and were then given 60 test trials. Each trial began with the raising of the guillotine door and the presentation of a 2,800 Hz tone. If the rat had not moved to the other side after 5 sec of the tone, a 1 mA scrambled shock was delivered to the floor of the compartment in which the rat was standing. Both the tone and the shock then remained on for 25 sec or until the animal crossed into the other compartment. At the same time the guillotine door was lowered. The inter-trial interval was 30 sec.An avoidance response was recorded if the animal moved to the other side of the shuttlebox during the 5 sec warning period preceding the shock.

RESULTS AND DISCUSSION

No attempt was made to quantify the overt signs of alcohol withdrawal in either the Alcohol Withdrawal or Continuous Alcohol groups although such signs were observed [5,10].

The results of the shuttlebox avoidance testing are shown in Fig. 1. A two-way analysis of variance with repeated

TABLE 1

MEANS ± STANDARD DEVIATIONS FOR ACTIVITY AND ESCAPE PERFORMANCE IN THE SHUTTLEBOX

Treatment	Activity*	†Escape Latencies
Alcohol Withdrawal	12.2 ± 4.1	1.6 ± 0.25
Continuous Alcohol	11.1 ± 3.2	1.4 ± 0.31
Sucrose Control	10.4 ± 5.6	1.3 ± 0.22

*Number of crossings during 5-min exploratory period.
†Sec from onset of shock.

measures on trials was carried out and yielded a significant groups effect, $F(2,28)=5.2$, $p<0.02$ an a significant trials effects, $F(5,140)= 120.2$, $p< 0.001$. The groups by trials interaction was not significant indicating a distinct deficit rather than a retardation of learning, i.e. all three groups reach an asymptote within 60 trials but at different levels of performance. Figure 1 shows that the Alcohol Withdrawal group made significantly fewer avoidance responses than either the Continuous Alcohol group or the Sucrose Control group. That this is a deficit in learning is supported by the fact that the three groups did not differ in activity, i.e. in the number of crossings during the 5-min. exploratory period, or in their sensitivity to pain, i.e. in the time they took to escape the shock (cf. Table 1).

During the liquid diet phase of the experiment the Alcohol Withdrawal group gained 28.8 g, the Continous Alcohol group 30.1 g and the Sucrose Control group 29.7 g. Further, the mean daily ethanol intakes of the Alcohol Withdrawal group and Continuous Alcohol group were similar, being 17.5 ± 2.4 g/kg and 15.3 ± 3.8 g/kg. respectively. As such, the observed deficit in shuttlebox avoidance cannot be attributed to malnutrition or differential ethanol intake in the Alcohol Withdrawal group. The most likely explanation is that repeated withdrawal from alcohol can, of itself, lead to an impairment of shuttlebox avoidance learning.

The present results extend the findings of Freund [5] in demonstrating that repeated alcohol withdrawal episodes can lead to an impairment of shuttlebox avoidance learning in rats. However, it remains to be seen whether a generalized learning deficit is established.

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